Prebiotics Reduce Body Fat and Alter Intestinal Microbiota in Children Who Are Overweight or With Obesity

Alissa C. Nicolucci,1 Megan P. Hume,1 Inés Martínez,2 Shyamchand Mayengbam,1 Jens Walter,2,3 and Raylene A. Reimer1,4

1Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada; 2Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada; 3Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada; 4Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada

BACKGROUND & AIDS: It might be possible to manipulate the intestinal microbiota with prebiotics or other agents to prevent or treat obesity. However, little is known about the ability of prebiotics to specifically modify gut microbiota in children with overweight/obesity or reduce body weight. We performed a randomized controlled trial to study the effects of prebiotics on body composition, markers of inflammation, bile acids in fecal samples, and composition of the intestinal microbiota in children with overweight or obesity. METHODS: We performed a single-center, double-blind, placebo-controlled trial of 2 separate cohorts (March 2014 and August 2014) at the University of Calgary in Canada. Participants included children, 7–12 years old, with overweight or obesity (>85th percentile of body mass index) but otherwise healthy. Participants were randomly assigned to groups given either oligofructose-enriched inulin (OI; 8 g/day; n=22) or maltodextrin placebo (isocaloric dose, controls; n=20) once daily for 16 weeks. Fat mass and lean mass were measured using dual-energy-x-ray absorptiometry. Height, weight, and waist circumference were measured at baseline and every 4 weeks thereafter. Blood samples were collected at baseline and 16 weeks, and analyzed for lipids, cytokines, lipopolysaccharide, and insulin. Fecal samples were collected at baseline and 16 weeks; bile acids were profiled using high-performance liquid chromatography and the composition of the microbiota was analyzed by 16S rRNA sequencing and quantitative polymerase chain reaction. The primary outcome was change in percent body fat from baseline to 16 weeks. RESULTS: After 16 weeks, children who consumed OI had significant decreases in body weight z-score (decrease of 3.1%), percent body fat (decrease of 2.4%), and percent trunk fat (decrease of 3.8%) compared with children given placebo (increase of 0.5%, increase of 0.05%, and decrease of 0.3%, respectively). Children who consumed OI also had a significant reduction in level of interleukin 6 from baseline (decrease of 15%) compared with the placebo group (increase of 25%). There was a significant decrease in serum triglycerides (decrease of 19%) in the OI group. Quantitative polymerase chain reaction showed a significant increase in Bifidobacterium spp. in the OI group compared with controls. 16S rRNA sequencing revealed significant increases in species of the genus Bifidobacterium and decreases in Bacteroides vulgatus within the group who consumed OI. In fecal samples, levels of primary bile acids increased in the placebo group but not in the OI group over the 16-week study period. CONCLUSIONS: In a placebo-controlled, randomized trial, we found a prebiotic (OI) to selectively alter the intestinal microbiota and significantly reduce body weight z-score, percent body fat, percent trunk fat, and serum level of interleukin 6 in children with overweight or obesity (Clinicaltrials.gov no: NCT02125955).

Keywords: Inulin-type Fructans; Pediatric Obesity; BMI; Adiposity.

The largest community of microbes in the human microbiota reside in the gut and, through a symbiotic relationship with the host, play a role in maintaining health and metabolic homeostasis, including the production of a diverse array of metabolites. Dysbiosis is associated with the promotion or aggravation of chronic metabolic diseases, including obesity and type 2 diabetes (T2D).1 One trigger for metabolic disease relates to the gut microbiota’s role in modulating inflammation whereby elevated circulating lipopolysaccharide (LPS), which is exacerbated by a high-fat or high-fructose diet, induces a low-grade inflammatory state termed metabolic endotoxemia.2–4 A shift in metabolite production is also observed with dysbiosis; this is particularly true for fecal bile acids (FBA), which require the gut microbiota for transformation.5 From a clinical stand point, there is great interest in determining if modulating the gut microbiota is a viable strategy to manage obesity and improve metabolic health.

Consumption of prebiotics, which are non-digestible food ingredients utilized by gut microorganisms and beneficially affect host physiology, is one such strategy.6,7 Microbial shifts in response to prebiotic intake have largely centered on changes in Bifidobacterium and

Abbreviations used in this paper: ANOSIM, analysis of similarities; BA, bile acid; BMI, body mass index; CDCA, chenodeoxycholic acid; FBA, fecal bile acid; FDR, false discovery rate; HOMA2-IR, homeostatic model assessment for insulin resistance 2; IL, interleukin; LPS, lipopolysaccharide; OI, oligofructose-enriched inulin; OTU, operational taxonomic unit; PCR, polymerase chain reaction; T2D, type 2 diabetes.
Lactobacillus, 2 common genera that may be increased with prebiotics and are associated with their beneficial effects on host health. However, with the understanding that global community structure and microbial diversity is important for intestinal and host health, there is a need to examine broader microbial changes that occur in response to prebiotics, for example with sequencing approaches. This approach is lacking in studies with children.

In a systematic review of clinical trials, prebiotic intake was associated with a significant improvement in satiety, postprandial glucose, and insulin concentrations in adult subjects. Consumption of an inulin/oligofructose blend has also been shown to increase Bifidobacterium spp. and Faecalibacterium prausnitzii, which both negatively correlated with LPS and Bifidobacterium spp. negatively correlated with percent fat mass and low-density lipoprotein cholesterol. These promising outcomes in adults justify the assessment of prebiotics as a dietary intervention to modulate gut microbiota and metabolic outcomes in pediatric obesity.

Excess weight in childhood tends to persist into adulthood and is an early risk factor for obesity-associated morbidity and mortality, highlighting the importance of early intervention. The potential for prebiotics to influence body weight in children was suggested by the slowed rate of weight gain observed in a trial assessing combined prebiotic and calcium intake in non-obese healthy children. To date, however, there is no research assessing the totality of changes in gut microbiota in children with overweight and obesity with prebiotic intervention. There is also limited research assessing global microbial composition of children with overweight and obesity or without an intervention. Therefore, our objective was to assess the effect of prebiotic supplementation on gut microbiota, FBAs, and associated metabolic outcomes (body composition, serum inflammatory markers, lipid profile, and fasting glucose and insulin concentrations) in otherwise healthy children with overweight and obesity.

Materials and Methods

Subjects
Male and female children, aged 7–12 years with overweight or obesity (≥ 85th body mass index (BMI) percentile) were voluntarily recruited from Calgary, Canada. This was a single centre, double-blind, placebo-controlled trial conducted in 2 separate cohorts (March 2014 and August 2014) at the University of Calgary. Following screening, subjects were randomly assigned using computer-generated numbers (and stratified according to age, sex, and BMI) to either prebiotic oligofructose-enriched inulin (OI) or placebo control maltodextrin for 16 weeks. The randomization was performed by an investigator that did not interact with the subjects, and 1 research assistant was responsible for all product distribution. Randomization sequences were not revealed to the study staff. Subjects and research staff were blinded to the treatments that were provided in identical foil packets. Parents/care givers completed a health and lifestyle questionnaire on behalf of the subjects to assess eligibility. Eligible subjects were otherwise healthy children with ≥ 85th BMI percentile at a Tanner developmental stage ≤ 3 (assessed by physical exam by a pediatric endocrinologist from the Alberta Children’s Hospital, Calgary, Canada). Exclusion criteria included type 1 or 2 diabetes, liver disease, cardiovascular abnormalities, supplement or medication use influencing appetite, weight or metabolism, currently following a weight loss diet, ≥ 3 kg weight loss 12 weeks before the initial test day, extreme changes in exercise intensity 4 weeks prior, or antibiotic use < 3 months prior.

This study, which was powered on the primary objective of reduction in percent body fat with 80% power and α = .05, required a minimum of 18 subjects per group. An additional 4 subjects were added per group to compensate for a potential 20% drop-out rate. Ethics approval was received from the Conjoint Health Research Ethics Board at the University of Calgary, REB13-0975. Written and informed consent was provided by the parents and verbal assent was provided by the subjects before the initial test day.

Dietary Intervention
Subjects were randomized to consume either 8 g/day (13.2 kcal/d) of OI, (Synergy1; BENEO GmbH, Mannheim, Germany) or an isocaloric dose of 3.3 g/day of maltodextrin placebo (Agenamalt 20.222; Agrana, Konstanz, Germany). Maltodextrin has a similar taste and appearance to Synergy1 and has been used previously in prebiotic trials. The prebiotic and placebo were consumed as a powder and provided to participants in pre-weighed individual packets. Participants and their parent(s) were instructed to dissolve an entire packet in 250 mL of water in a provided reusable water bottle. They were instructed to consume half the dose for the first 2 weeks, to promote adaptation and mitigate gastrointestinal symptoms, and the full dose for the remaining 14 weeks, 15–20 minutes before their evening meal. Empty and unused packets were returned to measure compliance. Our objective was to examine the effects of the prebiotic supplementation independent of any other lifestyle changes; therefore, subjects purchased their own food, were instructed to eat until comfortably full, and maintain their usual level of physical activity. An informal interview was
conducted at the end of the study to assess if subjects and their parents remained blinded throughout the study.

**Physical Characteristics and Body Composition**

Fat mass and lean mass were measured using whole-body dual-energy x-ray absorptiometry (DXA; Hologic QDR 4500; Hologic, Inc, Bedford, MA). Android and gynoid fat was estimated using the Hologic QDR software according to Arnberg et al. The android to gynoid fat ratio (A:G) was calculated as [android fat mass/gynoid fat mass]. Height, weight, and waist circumference were measured in duplicate at baseline and every 4 weeks thereafter. Height and weight z-scores were calculated using the Baylor College of Medicine-Body Composition Laboratory: Pediatric Body Composition Reference Charts online calculator. To track physical activity, subjects completed the Godin’s Leisure-Time Exercise Questionnaire at the initial, mid, and final test days.

**Blood Analysis**

A fasted blood sample was obtained at baseline and final test days. Serum lipids were analyzed by Calgary Lab Services (Calgary, Canada). Serum inflammatory cytokines were quantified using Human Adipokine Milliplex kits (Millipore, St. Charles, MO) and Luminex instrument at Eve Technologies (Calgary, AB, Canada). Plasma LPS was measured using the Pyro-Gene Recombinant Factor C Endotoxin Detection assay (Lonza Group Ltd, Basel, Switzerland) and fasted plasma glucose using the Glucose Trinder assay (Stanbio Laboratory, Calgary, AB, Canada). Primary PCRs amplified the V3-V4 region of the 16S rRNA gene and secondary PCRs attached dual indices to amplified regions with manufacturer recommended primers. Sequencing was performed with dual indexed paired 300 bps. Results were approximately 20 million total reads.

**FBA Analysis**

FBAs were profiled using high-performance liquid chromatography. Briefly, lyophilized, powdered fecal samples (10–20 mg) were suspended in water (250 μL) and heated for 10 minutes at 90°C. Samples were cooled then incubated for 16 hours at 37°C after adding 250 μL of sodium acetate buffer (100 mmol/L, pH 5.6) containing 15 units of cholylglycine hydrolase and 150 units of sulfatase. Isopropanol (500 μL) and 1 mol/L NaOH (100 μL) were then added and alkaline hydrolysis was performed by incubating 2.5 hours at 60°C. An internal standard (nordeoxycholic acid, 50 mmol/L) and 3 mL of 0.1 mol/L NaOH was added and FBAs were extracted through ultrasonication for 1 hour. After centrifugation, the supernatant was collected and cleaned using a Sep Pak T18 cartridge where the FBAs were eluted with 6 mL of methanol. The eluate was dried under Speedvac at 40°C. The unconjugated FBAs were derivatized by adding 150 μL of triethylamine (10 μL/mL) and 2-acetobromophenone (12 mg/mL) to their 24-phenacyl esters under ultrasonication for 1.5 hours at 50°C. The derivatized FBAs were further cleaned using a Sep-Pak silica cartridge and the eluate was dried under Speedvac at 30°C. The derivatized samples were suspended in 82% methanol and filtered through a 3 kDa centrifuge filter before injecting into the high-performance liquid chromatography. Individual bile acid (BA) 24-phenacyl esters were detected at 254 nm.

**Gut Bacterial Community Profiling–16S rRNA Quantitative Polymerase Chain Reaction**

Subjects and parent(s) were instructed to collect a stool sample preferably the evening before but up to 3 days before baseline and final test days using a stool collection kit. Stool was placed in a sterile conical tube and stored in a biohazard bag in the participant’s home freezer. Samples were brought to the laboratory on ice and stored at -80°C until analysis. Total bacterial DNA was extracted using FastDNA Spin Kit for Feces (MP Biomedicals, Lachine, QC, Canada) and quantified using the Nanodrop 2000 (Thermo Fisher Scientific Inc, Waltham, MA). All samples were diluted to 4 ng/μL before storage at -30°C. Amplification and detection was conducted in 96-well plates with SYBR Green qualitative polymerase chain reaction (qPCR) Master Mix (BioRad, Hercules, CA) according to our previous work using group specific primers.

**Statistical Analysis–Biological and qPCR Outcomes**

Data were presented as mean ± SEM. Analysis was performed on an intent-to-treat basis, regardless of subject compliance or completion. Cases with missing outcomes were excluded from analysis for that outcome. Normality was verified for each outcome and corresponding non-parametric tests were conducted on outcomes with a skewed distribution. Parametric tests were used to compare baseline measurements (independent t-test), between-group differences using mean
Clinical Authors approved the study design, and all interventions were carried out in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the institutional review board and registered with ClinicalTrials.gov (NCT01639587). All participants provided written informed consent at the time of enrollment.

**Results**

**Subject Characteristics**

A total of 42 subjects consented to participate in the study of which 22 were randomized to the prebiotic group and 20 to the placebo group. A total of 4 subjects withdrew for personal reasons (time constraints) or otherwise not specified (Supplementary Figure 1). Therefore, a total of 38 children, 20 in the prebiotic group and 18 in the control group, completed the study (90% retention). There were no significant differences in baseline characteristics between the groups (Table 1). A total of 81.8% of participants were white and 18.2% classified as “Other” (represented by black and Hispanic).

**Prebiotics Induced Marginal Changes in Systemic Inflammation**

There was little change in the serum inflammatory profile within the prebiotic and placebo groups (Supplementary Table 1). Although there was a 31% decrease in serum C-reactive protein in the prebiotic group and an 8% increase in the placebo group, this was not significantly different. Between-group analysis did show a significant reduction in interleukin (IL)-6 from baseline with placebo. Although absolute body weight increased in both groups over 16 weeks, the increase was significantly higher in placebo (2.4-fold greater weight gain) compared with prebiotic (Table 2). Age and sex-specific analysis of body weight showed significant decreases in body weight z-score within the prebiotic group ($P = .006$). The interaction of treatment and time significantly influenced BMI ($P = .009$) whereby there was no change in BMI within the prebiotic group compared with baseline, but at all 4 time points compared with baseline, BMI significantly increased in the placebo group (Supplementary Figure 2). Percent total body fat was significantly lower with OI compared with placebo ($P = .005$; Figure 1). Both groups had a significant increase in lean mass. Regional body fat assessment showed significant decreases in percent trunk fat within the prebiotic group ($P = .019$) and significant differences between the groups ($P = .029$). Percent android fat tended to be reduced in prebiotic vs placebo ($P = .055$).

**Metabolic Outcomes**

There was a significant decrease in serum triglycerides within the prebiotic group, but no between-group differences in lipid profile (Supplementary Table 2). There were no differences in fasting glucose, insulin, or HOMA2-IR within or between groups. At the end of the trial, however, 4 of the subjects in the prebiotic group (3 male, 1 female, baseline BMI 29.0 ± 2.9 kg/m², baseline percent total body fat 46.7 ± 2.2%, baseline trunk fat 46.7 ± 2.2%, baseline HOMA2-IR 2.5 ± 0.2), compared with zero in the placebo group, were no longer classified as insulin-resistant as defined by HOMA2-IR (HOMA2-IR > 2.10).28

**Fecal Bile Acids**

Primary FBAs, cholic acid and chenodeoxycholic acid (CDCA) were significantly different between the OI and placebo group after adjusting for age, sex, initial BMI, and compliance (Table 3). Within-group analysis showed significant increases in both primary FBAs in the placebo group, compared with baseline (Figure 2).
Table 2. Changes in Anthropometric Outcomes in Children (7–12 Years) With Overweight and Obesity Consuming OI (Prebiotic) or Placebo for 16 Weeks.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Prebiotics</th>
<th>Placebo</th>
<th>Initial</th>
<th>Final</th>
<th>Change</th>
<th>P value</th>
<th>Initial</th>
<th>Final</th>
<th>Change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td></td>
<td></td>
<td>14.1 ± 2.4</td>
<td>15.5 ± 2.4</td>
<td>&lt;0.001</td>
<td></td>
<td>1.31 ± 0.24</td>
<td>1.57 ± 0.24</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td></td>
<td></td>
<td>5.6 ± 3.1</td>
<td>6.6 ± 3.1</td>
<td>&lt;0.001</td>
<td></td>
<td>9.2 ± 0.10</td>
<td>10.2 ± 0.10</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-iliac crest (cm)</td>
<td></td>
<td></td>
<td>87.3 ± 2.1</td>
<td>92.0 ± 2.1</td>
<td>&lt;0.001</td>
<td></td>
<td>8.2 ± 0.22</td>
<td>9.2 ± 0.22</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-umbilicus (cm)</td>
<td></td>
<td></td>
<td>89.2 ± 2.1</td>
<td>92.6 ± 2.1</td>
<td>&lt;0.001</td>
<td></td>
<td>8.7 ± 0.22</td>
<td>9.7 ± 0.22</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td>26.2 ± 0.7</td>
<td>26.0 ± 0.7</td>
<td>&lt;0.001</td>
<td></td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Characterization of Gut Microbial Changes

Quantitative analysis of specific taxa (qPCR) showed a within-group difference of increased *Bifidobacterium* spp. in prebiotic (*P = .023*) and decreased *Clostridium* cluster XI (*P = .044*) in placebo (Supplementary Table 3). A significant between-group difference was seen for *Bifidobacterium* spp. with OI intake resulting in significantly higher abundance than placebo at 16 weeks (*P = .049*). The change in *Bifidobacterium* spp. from baseline was also significantly different between the prebiotic and placebo group (1.71% ± 0.80 vs 0.13% ± 0.94, *P = .049*).

A community-wide analysis with Illumina 16S rRNA sequencing mirrored the outcomes from qPCR (Table 4). OI consumption resulted in a significant bifidogenic response over the 16 weeks within the prebiotic group. Actinobacteria, the only observed phylum level change, significantly increased 1.4 fold (*P = .008, FDR=0.217*) and at the genus level *Bifidobacterium* abundance significantly increased. Moreover, 2 OTUs most likely representing *Bifidobacterium adolescentis* (OTU_2169) and *Bifidobacterium longum* (OTU_2403) significantly increased. Between-group analysis highlighted an interaction effect of treatment and time on *Bifidobacterium longum* (OTU_14) although not statistically significant (Supplementary Table 4).

Further analysis of within- and between-group differences showed significant changes in bacterial community composition beyond *Bifidobacterium*. Within-group analysis showed significant decreases in *Faecalibacterium prausnitzii* (OTU_2516) abundance with prebiotic (*P = .002, FDR=0.153*) (Table 4). The interaction between treatment and time significantly influenced 2 separate OTUs representing *F. prausnitzii* (OTU_2516 and OTU_1938) (Supplementary Table 4). Although no significant changes in the Bacteroidetes phylum or the genus *Bacteroides* were observed, *Bacteroides* sp. (OTU_29) was significantly influenced by the interaction of treatment and time. OI level analysis of within-group differences revealed OI significantly decreased *Bacteroides vulgatus* (OTU_2492, *P = .005, FDR=0.155*) (Table 4). Significant decreases in OTU_2376 representing *Ruminococcus gauvreaui* were also observed with consumption of OI, whereas decreases observed in genus *Ruminococcus* were not statistically significant following adjustment (*P = .026, FDR=0.311*).

The Shannon and Simpson Index, which were used to assess alpha-diversity, significantly decreased within both groups and no significant change in observed OTUs was detected within either group (Table 4). Beta-diversity, assessed using nonmetric multidimensional scaling plots based on Bray-Curtis distances, revealed differential clustering within the prebiotic group from baseline to 16 weeks.
determined using an ANOSIM ($P = .042$) (Supplemental Figure 2). However, there was no appreciable differential clustering of the data observed between the groups or within the placebo group.

**Microbial Correlations With Clinical Biomarkers**

Spearman’s correlation analysis was used to assess the relationship between changes in gut microbial abundance and changes in body composition and biological parameters (Figure 2). When assessing correlations with change in body composition, changes in body weight ($r_s = 0.414, P = .012$), fat mass ($r_s = 0.358, P = .032$), and BMI ($r_s = 0.373, P = .025$) were significantly and positively correlated with changes in OTU_2559 representing *Clostridium clostridioforme* and change in trunk body fat was significantly and positively correlated with change in *Bacteroides vulgatus* (OTU_2492) ($r_s = 0.494, P = .002$) and change in bacterium mpn-isolate (OTU_1554) ($r_s = 0.394, P = .017$). Change in OTU_2559 was also significantly and positively correlated with changes in IL-6 ($r_s = 0.657, P = .0001$), whereas change in serum triglycerides was significantly and positively correlated with

| Table 3. Changes in Fecal Bile Acids Assessed Using HPLC in Children (7–12 years) With Overweight and Obesity Consuming OI (Prebiotic) or Placebo for 16 Weeks$^a$ |
|-----------------------------------------------|----------|------------|-----------|----------|--------------|----------|-----------|-----------|
| Outcome ($\mu$mol/g, dry feces) | Prebiotics | Placebo | Prebiotics | Placebo | Prebiotics | Placebo | Prebiotics | Placebo |
| | Initial | Final | $P$ value | Initial | Final | $P$ value | Initial | Final | $P$ value |
| Primary Bile Acids | | | | | | | | | |
| CA | 1.651 ± 0.500 | 2.290 ± 0.949 | .967 | 1.801 ± 0.449 | 3.374 ± 1.533 | .007 | .043 |
| CDCA | 1.010 ± 0.437 | 1.246 ± 0.739 | .984 | 0.705 ± 0.293 | 2.539 ± 1.699 | .003 | .008 |
| Secondary Bile Acids | | | | | | | | | |
| DCA | 4.018 ± 0.918 | 6.464 ± 2.303 | .479 | 3.974 ± 0.885 | 7.797 ± 2.853 | .979 | .951 |
| iso-DCA | 9.372 ± 0.065 | 6.507 ± 0.131 | .898 | 3.360 ± 0.052 | 4.823 ± 0.095 | .985 | .940 |
| LCA | 4.694 ± 0.880 | 4.909 ± 1.115 | .574 | 3.842 ± 0.913 | 5.034 ± 1.105 | .247 | .202 |
| iso-LCA | 1.146 ± 0.229 | 0.895 ± 0.148 | .119 | 1.003 ± 0.170 | 1.103 ± 0.147 | .115 | .230 |
| HDCA | 0.344 ± 0.113 | 0.406 ± 0.082 | .697 | 0.383 ± 0.141 | 0.362 ± 0.041 | .759 | .500 |
| UDCA | 0.338 ± 0.102 | 0.516 ± 0.193 | .994 | 0.341 ± 0.092 | 0.450 ± 0.108 | .206 | .528 |
| CDCA% | 6.89 ± 2.32 | 5.97 ± 1.92 | .821 | 5.56 ± 1.77 | 6.50 ± 2.75 | .002 | .018 |

Abbreviations: OI, oligofructose-enriched inulin; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; HDCA, hyodeoxycholic acid; UDCA, ursodeoxycholic acid; CDCA%, proportion of CDCA to total fecal bile acids.

$^a$Values are mean ± SEM, n=16 for prebiotic and n=13 for placebo.
change in Ruminococcus gauvreaui (OTU_2376) ($r_s=0.479$, $P=.05$).

**Side Effects and Compliance**

No gastrointestinal side effects were experienced by 70% of participants in the prebiotic group and 61.1% in the placebo. A mild increase in flatulence and bloating was experienced by 25% and 27.8% of subjects in prebiotic and placebo, respectively. A moderate increase in flatulence and bloating was reported by 5% and 11.1% of subjects in prebiotic and placebo, respectively. During the informal interview to assess blinding, 50% of the prebiotic group and 72.2% of the placebo group were able to correctly guess their grouping. There was 87% and 91% compliance in the prebiotics and placebo group, respectively.

**Discussion**

This is the first randomized controlled study to assess the totality of changes in gut microbial composition and FBAs with prebiotic intervention in children with overweight and obesity. The results demonstrate that OI consumption normalizes childhood weight gain, reduces whole
body and trunk body fat, modifies primary FBAs, and selectively alters gut microbiota.

Although weight loss is a key outcome in obesity interventions, pediatric trials must consider the confounding effects of growth. One other trial with prebiotics in youth\(^2\) did not observe a reduction in absolute body weight with OI, which is consistent with our findings. However, height and weight are expected to increase linearly in children 6–10 years old with little difference between the sexes; in particular, body weight is expected to increase 2–3 kg annually.\(^3\) Based on our 4-month intervention data, the annual projected body weight increase in the prebiotic group would be 3 kg, within the expected range, whereas the projected increase in the placebo group was 8 kg, almost triple the expected yearly increase. The normalization of absolute body weight gain with OI is important because it allowed children to meet and not exceed expected growth trends.\(^2\) This normalization may be attributed in part to the improved appetite control we previously demonstrated in the OI group.\(^3\)

In the present study, the reduction in percent body fat observed in the subjects consuming OI was similarly observed by Abrams et al\(^1\) as a reduction in total fat mass in normal weight and overweight children consuming 8 g of OI with supplemental calcium for a year. Important from a metabolic health perspective,\(^2\) percent trunk fat was

---

**Figure 2.** Heat map of the Spearman rank correlations between biological and gut microbial outcomes. Correlations were performed on the change in outcomes over the 16-week intervention. \(^*P < .05\) and \(^†P < .01\). BF, body fat; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; IL, interleukin; TNF\(\alpha\), tumor necrosis factor alpha; UDCA, ursodeoxycholic acid; DCA, deoxycholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; HDCA, hyodeoxycholic acid; LCA, lithocholic acid; FA, fecal bile acids.
decreased in our participants consuming OI, which was similarly observed in adults with overweight and obesity consuming oligofructose for 12 weeks. The decrease in central adiposity in the present study could explain in part the significant reduction in serum triglycerides observed in the prebiotics group.

A proposed mechanistic link between obesity and its associated comorbidities, such as insulin resistance, is low-grade inflammation. Increased IL-6 and TNF-α are seen in adult obesity, while C-reactive protein is positively correlated with obesity in children and adults. In healthy, normal-weight adults, there was no change in cytokines following prebiotic intake, likely because baseline levels were not elevated enough to detect differences. This is likely the case in our subjects as well, given that IL-6 was the only cytokine significantly reduced with prebiotic and greater inflammation is typically needed to see a treatment response. We did observe a trend toward a reduction in metabolic endotoxemia, which is consistent with reduced LPS seen with inulin-type fructan intake in otherwise healthy obese adults, healthy normal weight adults, and overweight and obese women with T2D.

Microbial metabolites, such as FBAs, are 1 potential mechanism through which changes in gut microbiota composition impact host physiology. Increased primary FBAs have been associated with negative clinical outcomes, including diarrhea-prominent irritable bowel syndrome, which was associated with a significant decrease in Bifidobacterium. Of relevance to obesity, increased serum levels of the primary BA, CDCA relative to total BAs (CDCA%) was seen in obesity with T2D > obesity > overweight > healthy control. There was also a positive correlation between CDCA% and BMI, HbA1c, LDL-cholesterol, and triglycerides. In our participants, no change in fecal CDCA% was seen in the OI group (albeit a numerical but not significant decrease) but there was a significant 17% increase in the placebo group over time. It is possible that intake of OI mitigated the natural trajectory of increased primary FBAs seen in the placebo group, in part through increased Bifidobacterium.

Although our current understanding of what constitutes a healthy microbiota is still incomplete, certain genera have been established as primarily beneficial, including species in the genus Bifidobacterium. Infant studies highlight the benefits of increased bifidobacteria, with Bifidobacterium spp. dominating the gut of breast-fed infants that is associated with a reduced likelihood of overweight and obesity in childhood. Similarly, adults with obesity had a significant reduction in Bifidobacterium spp. compared with healthy weight. Our analysis of microbial changes at the genus level showed sequencing results that mirrored those with qPCR. Significant increases in Bifidobacterium spp. within the prebiotics group in this study was the only microbial change when assessed with qPCR, which was also observed in diverse adult cohorts consuming various prebiotics. Sequencing analysis in the present study also showed significant increases in OTUs representing Bifidobacterium such as Bifidobacterium longum (OTU_2403), which was similarly observed after prebiotic supplementation in women with obesity. In the adult human gut, significant increases in Bifidobacterium were observed after >10 g/day of short-chain fructooligosaccharides. In our pediatric population, we observed a significant bifidogenic response in the prebiotic group with a lower 8 g/day dose of OI.

The definition of prebiotic is currently a hotly debated issue, largely because of advancements in high-throughput sequencing showing changes beyond Bifidobacterium and Lactobacillus and the requirement for selective utilization. In accordance with this debate, changes in several other species and genera were observed with OI consumption, although the number remained limited, supporting a selective utilization argument. In the present study, a significant decrease in Bacteroides vulgatus (OTU_2492) was observed with OI consumption. Importantly, this reduction in B vulgaris was correlated with a reduction in percent trunk fat over the 16-week intervention. This positive correlation between B vulgaris and adiposity was also observed in women with obesity after prebiotic intervention. C clostridioforme has been defined as a pathogenic bacteria associated with serious and invasive human infection. In reference to metabolic disease, 2 metagenome projects observed C clostridioforme significantly enriched in patients with T2D compared with healthy controls. In the present study, Clostridium clostridioforme (OTU_2559) decreased in prebiotic vs placebo, and it was significantly positively correlated with changes in different biological and compositional outcomes.

Faecalibacterium prausnitzii is a prominent butyrate-producing bacterium that has been suggested to have an anti-inflammatory role in inflammatory bowel disease and has been negatively correlated with LPS in participants with obesity. In the present study, however, F prausnitzii (OTU_2516) significantly decreased with OI consumption. This result is consistent with a cross-sectional study in India showing increased F prausnitzii abundance in children with obesity compared with non-obese children with qPCR, and more recently with Illumina sequencing showing 20% higher abundance of F prausnitzii in Italian children with obesity compared with normal-weight, including a positive correlation of BMI z-score with Faecalibacterium. Conversely, in adult populations, prebiotic significantly increased F prausnitzii abundance, and these differences may be because of the cross-feeding interactions between bifidobacteria and F prausnitzii. In vitro analysis revealed that the relationship between bifidobacteria and F prausnitzii, in the presence of inulin-type fructans, could be commensal or competitive, and this relationship was dependent on the bifidobacterial strain and its capacity for prebiotic degradation. Functional differences between genetic phylotypes of F prausnitzii with different capacities for butyrate production have also been observed with lean individuals having a genetic variant with a more moderate capacity for butyrate production compared with individuals with obesity and T2D. In accordance with this, children with obesity had higher stool concentrations of butyrate compared with normal-weight controls.
There are some limitations to our study, including a reduced generalizability of our findings because of a primarily white and middle to high socioeconomic status of our participants. Our participants were also otherwise healthy overweight and obese children and, therefore, future studies should also include a pediatric population with greater metabolic dysfunction to more fully understand how genotype and environment affect the relationship between the host and the gut microbiome. Lastly, many children in the study did not have regular bowel movements; therefore, our stool collection at baseline and final test days could not be tightly controlled (eg, time of day, 24-hour collection), which could affect the concentrations of some fecal metabolites such as FBAs.

In conclusion, supplementation with OI improved obesity outcomes in children with overweight/obesity. Importantly, we have shown that OI induced specific gut bacterial shifts compared with placebo. The metabolic and microbial findings from this study provide a foundation for a larger clinical trial in the pediatric population. Prebiotics are inexpensive and non-invasive and, therefore, a plausible dietary intervention in the overweight and obese pediatric population.

**Supplementary Material**

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2017.05.055.

**References**

18. The Oxford Centre for Diabetes Endocrinology and Metabolism. HOMA calculator. Available at: https://www.dtu.ox.ac.uk/homacalculator/.


Authors names in bold designate shared co-first authorship.

Received April 6, 2017. Accepted May 30, 2017.

Reprint requests
Address reprint requests to: Raylene A. Reimer, PhD, RD, Faculty of Kinesiology, University of Calgary, 2500 University Dr. NW, Calgary, AB, Canada T2N 1N4. e-mail: reimer@ucalgary.ca; fax: 403-284-3553.

Acknowledgments
The authors would like to thank Kristine Lee, Faculty of Kinesiology, University of Calgary, for technical assistance. Shelly Wegener, Dr Richard Pon, and Dr Paul Gordon, University Core DNA Services and Alberta Children’s Hospital Research Institute (ACHRI) Centre for Health Genomics and Informatics at the University of Calgary, for their technical assistance and support with the 16S rRNA sequencing and analysis. This work was supported by grants from the BMO Financial Group Endowed Research Fund in Healthy Living, Alberta Children’s Hospital Research Institute and the Canadian Institutes of Health Research (MOP115076-1). The funding agencies had no role in the design of the study or preparation of this manuscript, and had no influence on the data collection, analysis, and interpretation or manuscript publication. The oligofructose-enriched inulin (Synergy1) was provided by Beneo (Mannheim, Germany).

Conflict of interest
R.A.R. previously held funding from Beneo, manufacturer of oligofructose-enriched inulin, for a project not related to the current work. The other authors disclose no conflicts.

Funding
This work was supported by grants from the BMO Financial Group Endowed Research Fund in Healthy Living, Alberta Children’s Hospital Foundation, Alberta Children’s Hospital Research Institute and the Canadian Institutes of Health Research (MOP115076-1). These agencies had no role in study design, data collection, analysis, and interpretation, or manuscript preparation. A.C.N. and M.P.H. received scholarship funding from the Alberta Children’s Hospital Research Institute. A.C.N. received scholarship funding from the Canadian Institutes of Health Research. S.M. is funded through an Eyes High Postdoctoral Fellowship and an Alberta-Innovates Health Solutions Postdoctoral Fellowship.